Chapter - 3

Material and Methods
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MATERIAL AND METHODS

3.1 Material

Scientific Name : Cenchrus ciliaris L.
Synonyms : Cenchrus glaucus C.R. Mudaliar & Sundararaj
Pennisetum ciliare (L.) Link


Common names: buffel grass, buffelgrass, foxtail buffalo grass, blue buffalo grass
african foxtail grass (English); bloubuffelgras (South Africa); cenchrus cilié
(French); Büffelgras (German); anjan grass , koluk katai, dhaman (India); pasto
buffel (Spanish); zacate buffel (Spanish - Mexico); capim-búfels (Portuguese -
Brazil).

Morphological description:
Extremely variable species, tufted (sometimes shortly rhizomatous) perennial, with
types ranging in habit from ascendant to erect, and branching culms from about 0.3-
2.0 m at maturity. Leaf blades linear, 2-13 mm wide and 3-30 cm long; green, blue
green to grey green in colour, scabrous, mostly glabrous, sometimes hairy at the base.
Panicle an erect or nodding, straw, grey or purple coloured, bristly, false spike, 2-15
cm long and 1-2.5 cm wide, with seed units or fascicles inserted along a zig-zag axis.
Each bur-like fascicle comprises a single spike let or cluster of 2-4 spike lets, 3.5-5
mm long surrounded by an involucres of bristles of various length up to 16 mm long;
bristles barbed and ( hairy, giving the fascicle an adhesive quality. 330,000-550,000
seed units/kg, or 900,000-2,000,000 caryopses/kg. Deep, strong, fibrous root system
to >2 m. (Plate 1)

Distribution:
Native to: Africa: Angola, Botswana, Egypt, Ethiopia, Ghana, Kenya, Libya, Malawi,
Mali, Morocco, Mozambique, Namibia, Niger, Nigeria, Senegal, Somalia, South
Africa, Sudan, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe.
Indian Ocean: Madagascar. Asia: Afghanistan, Djibouti, India, Iran, Iraq, Israel, Jordan, Oman, Pakistan, Saudi Arabia, Syria, Yemen.

Europe: Sicily.

Widely naturalised in sub-humid and semi-arid tropics and subtropics.

Different countries all over the world have produced their own cultivars of *Cenchrus ciliaris*. An attempt is made here to describe their characteristics or attributes. It illustrates the significance and utility of *Cenchrus* all over the world.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Country/date released</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>'American' (also USA or Common; T-4464, PI 153671, Grif 1618, Q4841)</td>
<td>USA (1949) Australia (1956)</td>
<td>A medium-short, early maturing, non-rhizomatous type, with fine stems, dense green foliage, and purple inflorescence, suitable for light to medium textured soils. Similar to Gayndah, but earlier flowering. Better seedling survival on acid soils than other cultivars. Selected for drought tolerance and forage production. Rapid growth from very early spring through late summer. Growth continues during hottest part of summer with brief showers. Fast recovery from grazing and drought. Susceptible to buffel grass blight (<em>Pyricularia grisea</em>).</td>
</tr>
<tr>
<td>'Bella' (CPI 48280)</td>
<td>Australia (1994)</td>
<td>Intermediate in height between the shorter 'American' and 'Gayndah' and more robust 'Biloela'. Long inflorescences, long, relatively narrow, bright green leaves; higher Na concentration (0.7%) in the leaves and later flowering than other varieties. Relatively few rhizomes compared with 'Biloela' and 'Molopo'. Adapted to sub-humid to semi-arid subtropical environments. Establishes well on clay soils. Better early-season growth than 'Biloela', 'American', 'Gayndah' and 'Molopo'. Well-grazed by sheep and better utilised than the coarser 'Biloela'. Resistant to <em>Pyricularia grisea</em> in Australia.</td>
</tr>
<tr>
<td>'Bergbuffel'</td>
<td>South Africa (2000)</td>
<td>From North West Province, South Africa. Tufted erect growth habit, taller type, rhizomatous, leaves green (grey-green in 'Molopo') and pointing upwards. Selected for palatability (more palatable than 'Molopo') and drought resistance. Widely adapted with good cold tolerance. Resistant to <em>Pyricularia grisea</em> in Australia.</td>
</tr>
<tr>
<td>Variety</td>
<td>Origin/Year</td>
<td>Description</td>
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<tr>
<td>'Biloela'</td>
<td>Australia (1955)</td>
<td>Institutional collection, Type D, from Mpwapwa, Tanzania. Later maturing, taller (to 1.5 m) more robust, rhizomatous type, with glaucous (greyish) leaves. Well adapted to sandy soils, but better adapted to heavier clays than shorter varieties. More tolerant of moderate salinity than other cultivars. Very drought tolerant. Susceptible to <em>Pyricularia grisea</em> in North America and Australia.</td>
</tr>
<tr>
<td>'Blue'</td>
<td>USA (1952)</td>
<td>Institutional collection from South Africa. An intermediate, shortly rhizomatous type with stems to 60 or 90 cm. Selected for early spring growth recovery (about 3 weeks ahead of 'American'), vigorous summer growth, high forage production, rapid plant expansion, drought tolerance, resistance to injury by leafhoppers and aphids, and tolerance to light frost (active end of season growth continues about 3 weeks longer than for 'American'). Grows well on clay soils. Relatively low seed production. Readily eaten by cattle as pasture or hay.</td>
</tr>
<tr>
<td>'Boorara'</td>
<td>Australia (1962)</td>
<td>Origin uncertain. Tall, moderately rhizomatous type, finer stemmed, leaves glaucous; leafier, and slightly later flowering than 'Biloela'. Susceptible to <em>Pyricularia grisea</em> in North America and Australia.</td>
</tr>
<tr>
<td>'Chipinga'</td>
<td>Zimbabwe (1950s)</td>
<td>From Zimbabwe (20°S, 1,100 m asl, rainfall 1,100 mm). Fine, leafy type.</td>
</tr>
<tr>
<td>'Gayndah'</td>
<td>Australia (1934)</td>
<td>Institutional collection from Kenya. Fine, medium-short, tufted, non-rhizomatous type, to 90 cm tall (commonly 30-60 cm), mid-season flowering (later than 'WA'), suitable for light to medium texturized soils. Better stock acceptance and more densely tillered than 'Biloela'. Tolerant of heavy grazing. Resistant to <em>Pyricularia grisea</em> in North America, but susceptible in Australia.</td>
</tr>
<tr>
<td>Variety</td>
<td>Origin</td>
<td>Year(s)</td>
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<tr>
<td>------------------</td>
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</tr>
<tr>
<td>'Higgins'</td>
<td>USA</td>
<td>1966, 1968</td>
</tr>
<tr>
<td>'Kalahari'</td>
<td>South Africa</td>
<td>1999</td>
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<tr>
<td>'Kongwa 531'</td>
<td>Tanzania</td>
<td>1960s</td>
</tr>
<tr>
<td>'Laredo'</td>
<td>USA</td>
<td>2000</td>
</tr>
<tr>
<td>'Lawes'</td>
<td>Australia</td>
<td>1962</td>
</tr>
<tr>
<td>'Llano'</td>
<td>USA</td>
<td>1977</td>
</tr>
<tr>
<td>Variety</td>
<td>Origin</td>
<td>Description</td>
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<tr>
<td>'Marwar Anjan'</td>
<td>India (1985)</td>
<td>Institutional collection from Australia. For arid and semi-arid parts of India. Tall, thick stemmed, erect, rhizomatous drought hardy perennial with broad, long, pendulous leaves that remain green up to maturity. Widely adapted with high tillering ability and good regeneration. Cut 2-3 times per year, yielding 7,000 kg of green fodder and 3,000 kg of dry matter per hectare under desert conditions.</td>
</tr>
<tr>
<td>'Mbalambala'</td>
<td>Kenya</td>
<td>From south-east Kenya (0°, 210 m asl). Multitilled, semi-prostrate type, forming broad tufts. Very palatable to cattle.</td>
</tr>
<tr>
<td>'Molopo'</td>
<td>South Africa (early 1950s)</td>
<td>From North West Province, South Africa (ca. 27°S, 800 m asl, rainfall ca 150mm). A little taller and more rhizomatous than 'Biloela', distinctly grey leaves and straw-coloured seed heads. Later flowering than 'Biloela' but earlier than 'Bella'. Cold tolerant and grows longer into the cool season. Well adapted to heavier soils. Good seed production if adequately N fertilised. Highly susceptible to Pyricularia grisea in North America, but largely resistant in Australia.</td>
</tr>
<tr>
<td>'Mopani'</td>
<td>South Africa (1999)</td>
<td>Taller type, rhizomatous, leafier and with less dormancy than 'Kalahari', but not as cold tolerant or as vigorous, good seed production. Resistant to Pyricularia grisea in Australia.</td>
</tr>
<tr>
<td>'Nueces'</td>
<td>USA (1977)</td>
<td>Apomictic F1 hybrid between 'Higgins' and a rhizomatous introduction from Africa. Improved cold tolerance, better forage production, and higher IVDMD than 'American' and 'Higgins'. Highly susceptible to buffel grass blight in North America.</td>
</tr>
<tr>
<td>'Nunbank'</td>
<td>Australia (1961)</td>
<td>Institutional collection from Uganda. Similar to 'Biloela' in most respects, but has superior seedling vigour. Susceptible to Pyricularia grisea in North America and Australia.</td>
</tr>
<tr>
<td>Variety</td>
<td>Origin</td>
<td>Description</td>
</tr>
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</tr>
<tr>
<td>'Pecos'</td>
<td>USA (2000)</td>
<td>Mixture of several lines. More cold tolerant and more productive (30%) than 'American'. More cold hardy than 'Laredo'. Resistant to buffel grass blight in North America. Marketed by Pogue Agri Partners Inc.</td>
</tr>
<tr>
<td>'Sebungwe'</td>
<td>Zimbabwe</td>
<td>Dwarf strain, suitable for semi-arid conditions.</td>
</tr>
<tr>
<td>'Tarewinnabar'</td>
<td>Australia (1962)</td>
<td>From Kenya. Similar to 'Biloela' in most respects, except taller and leaves are green not glaucous. Good seedling vigour. Better early-season growth and slightly later flowering than 'Biloela'. More flood tolerant than other varieties tested. Susceptible to <em>Pyricularia grisea</em> in Australia.</td>
</tr>
<tr>
<td>'Viva'</td>
<td>Australia (1994)</td>
<td>From Moroto, Uganda (2°30'N, 1,400 m asl, rainfall ca 1,000 mm). Similar height to 'Gayndah', and shorter than 'Bella'. Green leaves; unlike 'Bella', it has a low leaf sodium concentration. Late flowering. Better early season growth than 'Biloela', 'American', 'Gayndah' and 'Molopo'. Few short rhizomes. Competes well with weeds during establishment. Well grazed by sheep. Resistant to <em>Pyricularia grisea</em> in Paraguay and Australia.</td>
</tr>
<tr>
<td>'West Australian'</td>
<td>Australia</td>
<td>Inadvertent introduction in 1870s. Non-rhizomatous, short tussock type to 75 cm (lowest growing of Australian varieties), with dense, fine, leafy tillers; leaves green. Very early flowering and not as vigorous as the taller varieties. Well suited to lower rainfall and short wet season areas because of earlier seed set. Good growth habit for sheep grazing. Higher intake and feeding value than 'Biloela'. Resistant to <em>Pyricularia grisea</em> in North America but susceptible in Australia.</td>
</tr>
<tr>
<td>'Worcester'</td>
<td>South Africa</td>
<td>Similar to 'Molopo' and 'Mopani', excellent seed production.</td>
</tr>
<tr>
<td>'Zeerust'</td>
<td>South Africa (1940s)</td>
<td>Tall, leafy form adapted to 500-625 mm rainfall area in South Africa.</td>
</tr>
</tbody>
</table>

Source: Mandy Tu, (2002)
*Dichanthium annulatum* Stapf. (Apang, Marvel) is widespread in India, Burma, Tropical and North Africa. It is readily recognized by the presence of a hairy ring on the node and a cluster of 4 or more scarlet coloured earheads. It is very palatable and contains about 5 per cent protein. It also makes a very fine hay. The grass is suitable for both light and heavy well drained soils. The grass can be established by seeds or rooted plants during monsoon.

*Sehima nervosum* stapf. is widespread in South East Asia, East Africa and Australia. This is often subgregarious and is considered to be an excellent fodder grass. It is commonly known as Rat's tall grass, white grass (Northern Territory, Australia) It is Annual or perennial, culms densely tufted with leaf-blades up to 30 cm long. It survives the long dry season in northern Australia and dry seasons in India very well. It grows well on loamy sands with a pH of 6.5 in India, but grows best on black soils.
3.2 To study seed storage and viability of *Cenchrus ciliaris*.

Seed storage of any plant is a way of germplasm preservation and vital for all involved and interested in seeds. *Cenchrus ciliaris* seeds that were freshly harvested in 2005 at CAZRI were stored under two different temperature conditions for the period of three years. Certified seeds of *C. ciliaris* were procured from CAZRI (Central Arid Zone Research Institute), Jodhpur, Rajasthan.

Detailed study was taken up to assess the storability of seeds of *Cenchrus ciliaris* as a function of different temperature conditions by assessment of:

(a) germination percentage; (b) seedling morphological parameters.

After determination of initial seed moisture content the seeds were sealed in polythene bags (700 gauge thick). The sealed bags were stored at two different temperatures. (1) Room temperature (20±2°C) (2) Cold temperature (-15±2°C) for the period of three years. The stored seeds were sampled annually for two years and tri-monthly interval for the third year. Various parameters recorded (ISTA, 1985) are as follows:

1. **Seed moisture content**: The seed moisture content as percentage was obtained from the following formula:

   \[
   \text{% moisture content} = \frac{W_1 - W_2 \times 100}{W_1}
   \]

   Whereas many biologists may calculate it on a dry weight basis, i.e.,

   \[
   \text{% moisture content} = \frac{W_1 - W_2 \times 100}{W_2}
   \]

   Where \( W_1 \) = Pre-drying seed weight.

   \( W_2 \) = Post drying weight after drying at 80 degree centigrade for 48 hours.

2. **Germination percentage**: Percentage germination was calculated based on normal seedling growth recorded on 7th day as follows:

\[
\text{S/T} \times 100
\]

\( S \) = number of normal seedlings.

\( T \) = Total number of seeds kept for germination.
Two replicates of 50 seeds each were raised by the following method of germination: Paper Top Method: Seeds were surface sterilized with 0.1% HgCl₂ and placed in a Petriplate and lined with two layers of moist filter paper and watered with 5 ml of distilled water (ISTA, 1985). The plates were covered to restrict moisture loss and kept at room temperature for seven days. Germinated seeds were removed and counted on the 7th day of germination.

3. **Seedling length**: Length of ten seedlings was measured on 7th day of germination. Shoot length and root length were measured separately. Total length of seedling was compared. Data presented are based on average values of ten seedlings from each replicate. The whole experiment was repeated thrice.

4. **Seedling Fresh and Dry weight**: After recording seedling fresh weight, same seedlings were packed in blotting paper and kept in an air-oven maintained at 80±2°C till constant weights were recorded. Data presented are based on average values of ten seedlings from each replicate. The whole experiment was repeated thrice.

5. **Seedling Vigour Index (SVI)**: Vigour indices were calculated by multiple methods as suggested by Abdul Baki and Anderson (1973).

\[
\text{SVI-D} = \% \text{Germination} \times \text{Dry wt. of seedling}
\]

\[
\text{SVI-L} = \% \text{Germination} \times \text{Total Seedling Length}
\]

Soil Analysis was done before the experiments to know the status of soil at GEER Foundation, Gandhinagar.

3.3 **Methods of soil analysis** (B.K. Sharma, 2002)

Before starting field experiments at GEER Foundation soil analysis was done to obtain the soil status.

**pH**:

pH of soil is the measure of hydrogen ion activity and depends largely on the relative amounts of adsorbed hydrogen and metallic ions. It is a good measure of the intensity of acidity and alkalinity of soil-water suspension, and provides a good identification of the soil chemical nature.
pH of soil suspension highly depends on the soil water ratios. In this method (1:2.5 soil water ratio) 20 g of soil and 50 ml of distilled water is taken and stirred for 30 minutes. Occasionally the electrodes of pH meter are dipped in suspension and reading on the scale is noted and soils are characterized accordingly.

**Electrical conductivity:**
Conductivity, as the measure of current carrying capacity, gives a clear idea of the soluble salts present in the soil. Like water the conductivity of the soil suspension is also measured by conductivity meter. Electrical conductivity or conductance is the reverse of resistance $= 1/R = 1/\text{ohm}$. It is expressed in reciprocal of ohm or mhols per cm. In laboratory it is determined with conductivity meters calibrated to read directly the electrical conductance with the given cell. The soil suspension or paste is taken in a conductivity cell for determination of electrical conductance.

**Available nitrogen**
A known weight of soil is mixed excess of alkaline KMnO₄ and Ammonia gas formed is absorbed in a known volume of standard sulphuric acid excess of which is titrated with standard alkali sodium hydroxide using methyl red as indicator. Available nitrogen in the soil is interpreted as follows.

- Below 224 kg/ha- very low
- 224 to 280 kg/ha- low
- 280 to 560 kg/ha – medium
- Above 560 kg/ha – high

**Available phosphorus**
Phosphorus in soils is generally determined as available Phosphorus. Soil $P_2O_5$ is determined by shaking the soil with N/2 sodium bicarbonate solution adjusted to pH 8.5. The dissolved phosphorus makes the phosphomolybate complex with ammonium molybate, which is reduced by stannous chloride to molybdenum blue giving colour which is determined colorimetrically. Results of the available $P_2O_5$ in the soil are interpreted as follows.

- Below 22.5 kg $P_2O_5$/ ha – low supply
- 22.5 to 56 kg $P_2O_5$/ ha – medium supply
- Above 56 kg $P_2O_5$/ ha – high supply
Available potash

The available K\textsubscript{2}O is extracted from the soil by shaking it with 1 N ammonium acetate solution adjusted to pH 7.0. The extracted Potassium is determined with Flame Photometer. The amount in soil is interpreted as follows:

- Below 140 kg/ha – low supply
- 140-336 kg/ha - medium supply
- Above 336 kg/ha – high supply

3.4 Study of the dynamics of mixed cropping of Cenchrus ciliaris with Dichanthium annulatum and Sehima nervosum.

The experimental plots were laid at the Botanical Garden of GEER Foundation, Gandhinagar (Gujarat). Three palatable fodder grasses which were selected for the experiment were *Cenchrus ciliaris, Dichanthium annulatum* and *Sehima nervosum*. Tussocks of *Cenchrus ciliaris* and *Dichanthium annulatum* were collected from Main Fodder Research Centre, Anand. *Sehima nervosum* was collected from Grassland Research Centre, Dhari. Seven plots of 3 mt. x 3 mt. size were prepared and the distance between the adjacent plots was 2 ft. The three grasses were sown individually and in combination of two and all three in separate plots. The tussocks were planted according to row method. Routine agronomic practices of fertilizer and irrigation were followed. The meteorological data namely average maximum temperature, minimum temperature, humidity and photoperiod were noted during the field experiment. Ten plants were randomly selected and growth data was recorded at fifteen days interval from all sets. The growth data included number of leaves and tillers, height of shoot and root, fresh weight of root, shoot and leaves. Above ground biomass productivity was calculated from the above ground fresh weight of leaves, tillers and shoots.

3.5 Study of effect of different concentrations and Combinations of fertilizers on growth, flowering and biomass production of Cenchrus ciliaris.

The experimental material consisted of *Cenchrus ciliaris* 358. The seeds of *C. ciliaris* were collected from Central Arid Zone Research Institute (CAZRI), Jodhpur. The material was sown in the experimental plots laid down at the Botanical
garden, GEER Foundation, Gandhinagar (Gujarat) in randomized block design with three replications. The Plots size was 3m×3m and distance between the adjacent plots was 2 ft. Row-to-row spacing was 30 cm and plant to plant spacing was 25 cm. The seeds were sown by row method. Three fertilizers selected for application are as follows:

1. Urea - 46% Nitrogen
2. Superphosphate - (16% P₂O₅, Total phosphate-17.6%, sulphur-11%, calcium-19%)
3. Muriate of potash - 60% K₂O.

The plots were fertilized with different concentrations of these three fertilizers separately and in combinations. Muriate of potash was applied in 2.5 to 5 cm wide bands on each side of the row of seeds at a depth of 10 cm, while Superphosphate and urea were applied as a top dressing. Urea was applied after fifteen days of sowing as it is harmful to seeds if applied before sowing. The application of fertilizers was followed by watering. The treatment was repeated after one month.

The different treatments are given below:

T1. Urea-200kg/ha
T2. Urea-400kg/ha
T3. Superphosphate-130kg/ha
T4. Superphosphate-260kg/ha
T5. Muriate of potash-130kg/ha
T6. Muriate of potash-260kg/ha
T7. Urea-400kg/ha + Superphosphate-260kg/ha
T8. Urea-400kg/ha + Muriate of potash-260kg/ha
T9. Superphosphate-260kg/ha + Muriate of potash-260kg/ha
T10. Urea-400kg/ha + Superphosphate-260kg/ha + Muriate of potash-260kg/ha

Regular irrigation practice was followed. Manual hoeing was done regularly to remove the weeds. Ten plants were randomly selected and growth data was recorded thrice at 20 days interval from all sets.

The data taken is as given below:
1. Height of the plant.
2. No. of tillers/plant.
3. Length and breadth of leaves.
4. Length of the root.
5. Above ground fresh wt.
For each treatment the number of tillers of individual plant were counted. The maximum roots generally occur in upper 30cm, depth of soil. Therefore, from the base of each plant 20cm, radius was formed and each plant was excavated upto 30cm, depth with ball of earth with a shawal. The individual plants were kept in polythene bags and labeled. Belowground biomass was assessed by washing thereby the excavated roots with a fine jet of water to remove the soil particles. The shoot portion was clipped upto ground level and green weight recorded in grams. This was recorded as fresh above ground biomass and fresh below ground biomass. All the above ground and below ground samples so collected were dried in oven at 80°C till the weight remained constant. The oven dried weight of shoot and roots were recorded in grams by using electronic balance. This was recorded as Dry- above ground biomass and Dry- below ground biomass respectively. Relative Growth Rate (RGR) was also calculated on the basis of above ground biomass (dry weight) as follows:

$$\text{RGR} = \log_e W_t - \log_e W_0$$

Where $W_t$= final dry weight of whole plant
$W_0$= initial dry weight of whole plant
$t$ = period of time

Meteorological variables, viz. rainfall, temperature (maximum and minimum), humidity, Photoperiod in hours and evaporation, were also recorded. Statistical analysis of the 60 days data was done using Dunnett t (2 sided) test and ANOVA to evaluate the significance of the treatments given.

3.6 Study of effect of different seasons on biomass production of Cenchrus ciliaris under Gandhinagar conditions.

The experimental material consisted of Cenchrus ciliaris 358. The seeds of C. ciliaris were collected from Central Arid Zone Research Institute (CAZRI),
Jodhpur. The material was sown in different seasons, Summer (February-May), Monssoon (June-September) and Winter (October-January) in the experimental plots laid down at the Botanical garden, GEER Foundation, Gandhinagar (Gujarat) in randomized block design with three replications. The plots size was 3m×3m and distance between the adjacent plots was 2 ft. Row-to-row spacing was 30 cm and plant to plant spacing was 25 cm. The seeds were sown by row method. Routine agronomic practices of fertilizer and irrigation were followed. The meteorological data namely average maximum temperature, minimum temperature, humidity and photoperiod were noted during the field experiment. Ten plants were randomly selected and growth data was recorded at monthly interval from all sets. The growth data included height of shoot, number of tillers, fresh weight of shoot and fresh weight of root. Above ground biomass was calculated from the above ground fresh weight of leaves, tillers and shoots. The maximum roots generally occur in upper 30 cm, depth of soil. Therefore, from the base of each plant 20 cm, radius was formed and each plant was excavated up to 30 cm, depth with ball of earth with a shawal. The individual plants were kept in polythene bags and labeled. Belowground biomass was assessed by washing thereby the excavated roots with a fine jet of water to remove the soil particles. The shoot portion was clipped up to ground level and green weight recorded in grams. This was recorded as fresh above ground biomass and fresh below ground biomass. The weight of all the above ground and below ground samples so collected were recorded in grams by using electronic balance. Statistical analysis of the seasonal data was done to evaluate the seasonal difference in biomass production.

3.7 Study of effect of clippings on overall biomass production of Cenchrus ciliaris

The experiment was conducted at the Botanical garden, GEER Foundation, Gandhinagar (Gujarat). 5 sub plots of 3m×3m size were prepared and distance between the adjacent plots was 2 ft.

In all plots the density and spacing of plants were kept uniform. The experiment was conducted in three replicates. One sub plot was left unclipped and other four were clipped. The clipping heights were (base, 5 cm, 10 cm, 15 cm). Clipping interval was 15 days, 30 days and 45 days. Frequency of clipping was 15 days (6 times), 30 days (3 times) and 45 days (2 times). Regular irrigation practices
were followed. Herbage production for each treatment was calculated after 90 days. For each treatment (1) Shoot length (2) No: of tillers (3) Above ground fresh weight (4) Below ground fresh weight were assessed. For each treatment the belowground biomass was assessed at the end of the experiment by digging the trench and washing thereby the excavated roots with a fine jet of water to remove the soil particles. The shoot portion was clipped upto ground level and green weight recorded in grams. This was recorded as fresh above ground biomass and fresh below ground biomass. The weight of all the above ground and below ground samples so collected were recorded in grams by using electronic balance. Statistical analysis of the 45- interval clipping data was done to evaluate the significance of the treatments.

3.8 Study of physiological (nutritional) changes in above ground biomass of Cenchrus ciliaris by using different concentrations and combinations of fertilizers.

Measurements of nutritive value were made in two replicates. The samples were oven-dried and ground through a 0.2 mm screen. Analyses using duplicate samples, of Crude Protein concentration, Nitrogen, Phosphorus, Potassium, Crude fibre, Silica was done. Concentration of Crude Protein (N concentration ×6.25) was estimated by a conventional microkjeldhal method. Following are the details of the methods.

Estimation of Protein

1. Scope and Field of Application

This method is for the determination of protein in feeding stuffs by the Kjeldahl method for nitrogen. (GAFTA. 1995)

2. Principle

The sample is digested by mineral acid. The acidic solution is made alkaline by a sodium hydroxide solution. The ammonia released is removed by distillation and collected in a measured quantity of sulphuric acid, the excess of which is titrated with a solution of sodium hydroxide.
3. **Reagents**

3.1 Catalyst: mercuric oxide.

3.2 Potassium sulphate or anhydrous sodium sulphate.

3.3 Sucrose.

3.4 Zinc, granulated.

3.5 Pumice stone, granulated, washed in hydrochloric acid and ignited.

3.6 Sulphuric acid (d=1.84g/ml).

3.7 Sodium hydroxide solution, carbonate free: dissolve 400g sodium hydroxide in water and dilute to 1, 000ml.

3.8 Sodium sulphide, cold saturated solution.

3.9 Sodium thiosulphate solution: 8g sodium thiosulphate (Na$_2$S$_2$O$_2$. 5H$_2$O) per 100 ml.

3.10 Sodium hydroxide solution, 0.1N,

3.11 Sodium hydroxide solution, 0.25N.

3.12 Sulphuric acid solution, 0.1N.

3.13 Sulphuric acid solution, 0.5N.

3.14 Methyl red indicator solution: dissolve 0.3g methyl red in 100ml of ethanol (95-96 % V/V).

3.15 Screened methyl red indicator solution:

(a) dissolve 0.2g methyl red in 100ml of ethanol (95-96% V/V).

(b) Dissolve 0.1g methylene blue in 100ml of ethanol (95-96 % V/V).

Mix one volume of (a) with one volume of (b).

4. **Apparatus**

Apparatus for mineral acid digestion and distillation according to Kjeldhahl's method.
5. **Procedure**

5.1 **Mineral acid digestion**

Material was weighed to the nearest 0.001 g. Approximately 1g of the prepared sample was transferred to a Kjeldahl Flask. 10g Potassium sulphate (3.2) or sodium sulphate (3.2), 0.6 to 0.7g mercuric oxide (3.1), 25 ml, sulphuric acid (3.6) and a few grains of pumice stone (3.5) were mixed and heated in the flask moderately at first, shaking from time to time, until the mass is carbonized and the froth was disappeared; then heat was increased and the liquid was brought to a steady boil. Overheating was avoided which may cause organic particles to stick to the sides of the flask. When the solution appeared clear and colourless boiling was continued for a further 2 hours and then was allowed to cool.

*Note:* If after the digestion and cooling crystallization occurs the analysis was repeated. If crystallization still occurs, the analysis was repeated using a larger quantity of sulphuric acid.

5.2 **Distillation**

250-350ml water was added carefully mixing the contents during the addition; which was then allowed to cool. Then a few pieces of zinc (3.4) were added.

25.0ml of 0.1N (3.12) or 0.5N Sulphuric acid (3.13) was transferred to the collecting flask of the distillation apparatus, according to the presumed level of nitrogen and a few drops of methyl red indicator (3.14) or (3.15).

Taking precautions against loss of ammonia, 100ml, sodium hydroxide solution (3.7) was added and then either 10ml, sodium sulphide solution (3.8) or 25ml sodium thiosulphate solution (3.9) was added .It was mixed well and connected immediately to the distillation apparatus.

Heat the flask so that approximately 150ml of the liquid is distilled in 30 minutes. At the end of this time, check the pH of the resulting distillate with indicator paper. If the reaction is alkaline, continue the distillation. Discontinue distillate with indicator paper. If the reaction is alkaline, continue the distillation. Discontinue distillation when the distillate appears neutral to
indicator paper, during the distilling process, swirl the contents of the collection flask from time to time.

Note: If the contents of collecting flask become alkaline, the determination should be abandoned and the analysis repeated making appropriate adjustments.

5.3 Titration

In the collection flask the excess sulphuric acid was titrated with sodium hydroxide solution 0.1N (3.10) or 0.25N (3.11) according to the normality of the sulphuric acid employed, to the end point of the indicator, (3.14) or (3.15).

6. Expression of the Results

The volume of sulphuric acid consumed was determined. 1ml 0.1N sulphuric acid = 1.4 mg nitrogen.

The percentage of nitrogen in the sample was calculated and converted to percentage protein by multiplying the result by 6.25.

Estimation of Phosphorus as P by Spectrophotometric Method

Principle: The sample is ashed in muffle furnace and the ash is extracted with acid. Phosphorus is estimated in the acid solution using Vanadomolybdate Spectrophotometric Method.

Reagents Required: -

1. Molybdovanadate reagent: Accurately weigh 0.47 g of ammonium vanadate into a 250ml beaker. Dissolve in distilled water. Accurately weigh 20 g of ammonium molybdate into a 400 ml beaker. Dissolve in distilled water. Mix both the reagents and acidify the clear solution with 140 ml of nitric acid. Dilute to 1000 ml and transfer to a reagent bottle and thoroughly mix.

2. Phosphorus Stock Standard: 1ml = 1mg P. Potassium dihydrogen phosphate, AR, 99.5% Pure, \( \text{KH}_2\text{PO}_4 \), M. Wt. 136.09. P = 22.765%. Dry in an oven at 105 °C for 1h. Accurately weigh 2.1969g into a funnel fitted to 500 ml volumetric flask and dilute to the mark.
3. Phosphorus Working Standard: 1 ml = 0.1 mg. Dilute 50 ml of Stock solution to 500 ml and make up to the mark.

Method: The silica crucible was thoroughly cleaned with dilute HCl, tap water followed by distilled water and was kept in oven for 15 minutes and later removed from the oven and allowed to cool. 2 g of the sample was accurately weighed into the crucible. 1 g of CaCO$_3$ was added to the sample and thoroughly mixed was introduced into the muffle furnace below 500°C and maintained at 500-600°C for 3h. and then removed from the furnace and allowed to cool to room temperature. The ash was transferred to 250 ml beaker. 10 ml distilled water, 10 ml Conc. HCl and 10 ml Conc. HNO$_3$ were added and evaporated to dryness. 10 ml HNO$_3$ was added and slightly warmed to dissolve, 50 ml distilled water was added after the residue was thoroughly gone into HNO$_3$ then boiled and filtered into a 250 ml volumetric flask. The residue was washed and made up to the mark. A blank was prepared using CaCO$_3$ without sample. The process of ignition and acid extract for the blank is same as the sample.

1.0 Nesslers Cylinder - 1 (Sample): Pipette 10 ml of the filtrate into a nesslers cylinder and add 25 ml distilled water followed by 10 ml of ammonium molybdovanadate reagent. Shake well and makeup to 50 ml mark.

2.0 Nesslers Cylinder - 2 (Standard): Take 20 ml distilled water and add 2 ml nitric acid. Shake well and add 5 ml working standard followed by 10 ml of Molybdovanadate reagent. Shake well and make up to 50 ml mark.

3.0 Nesslers Cylinder - 3 (blank): Pipette 10 ml of blank filtrate into a Nesslers cylinder and add 25 ml distilled water followed by 10 ml of ammonium molybdovanadate reagent. Shake well and make up to 50 ml mark.

Read the absorbance at 430 nm using blank (tube - 3) for making zero absorbance.

Calculations:

mg of "P" in 5 ml working standard = 0.5mg.

mg of sample in the 10 ml aliquot = \( \frac{10 \times 2000}{250} = 80 \) mg

\% Phosphorus as P = \( \frac{\text{Sample Absorbance} \times \text{mg P in 5 ml working STD}}{\text{mg sample in aliquot}} \times 100 \)

\% Phosphorus as P = \( \frac{\text{Sample Absorbance} \times 0.5 \times 100}{\text{Standard Absorbance} \times 80} \)
Estimation of Potassium as K by Using Flame Photometer

Principle: The sample is ashed in muffle furnace and the ash is extracted with acid. Potassium is estimated in the acid solution using Flame Photometer.

Reagents Required:

1. Use Double distilled Water for rinsing the glass ware and all the Process of Alkalies estimation
2. Conc. HNO₃
3. Stock Standard for Na and K (1000ppm mixed standard): Take a clean and dry watch glass. Place few grams of AR – NaCl in it and dry in the oven for few hours. Similarly dry AR - KCl also. Take a 1000 ml volumetric flask fitted with a funnel. Wash with double distilled water. Accurately weigh and transfer 2.542 g of NaCl (Mol. Wt 58.45, 99.9%) and 1.9066g of KCl (Mol. Wt. 74.55, 99.8%) into the funnel. Dissolve the salt and makeup to the mark and shake well.
4. Working Standard (20 ppm Na and K): Take a clean 500ml volumetric flask. Accurately pipette 10 ml of 1000 ppm standard and makeup to the mark.

Method: The silica crucible was thoroughly cleaned with dilute HCl, tap water was followed by distilled water. It was kept in an oven for 15 min and then was removed from the oven and was allowed to cool. 2.0000g of the sample was accurately weighed into the crucible. It was introduced into the muffle furnace below 500°C and maintained at 500-600°C for 3h and then was removed from the furnace and allowed to cool to room temperature. The ash was transferred to 250 ml beaker. 10 ml distilled water, 10 ml Con HCl and 10ml Con HNO₃ were added and evaporated to dryness. A 10ml HNO₃ was added and was slightly warmed to dissolve. 50ml distilled water was added after the residue is thoroughly gone into HNO₃ and then boiled and filtered into a 250ml volumetric flask. The residue was washed and made up to the mark. Blank was prepared using the same amount of acids as in sample omitting ignition step. Both samples and standard were diluted 10 times. 25 ml of above solutions were taken with Pipette separately into 250ml volumetric flasks and made up to the mark. The air Compressor and Gas were switched on and the flame was ignited. Distilled water was feeded. The instrument was allowed to stabilize for 30 minutes. The Potassium filter was fixed. The Zero reading was adjusted using the blank solution. 100 reading was adjusted using 20ppm standard.
Calculation:

\[
\% K = \frac{\text{Instrument Reading} \times 1 \times 94 \times 100}{5 \times 4 \times 78 \times 200}
\]

**Ash insoluble in hydrochloric acid (silica)**

1. Scope and Field of Application

This method is for the determination of hydrochloric acid-insoluble mineral substances in feeding stuffs.

2. Principle

2.1 Method: The sample is ashed, boiled in hydrochloric acid and the insoluble residue filtered and weighed.

3. Reagents

3.1 Hydrochloric acid solution, 3N.

3.2 Trichloroacetic acid solution, 20g per 100ml.

3.3 Trichloroacetic acid solution, 1g per 100ml.

4. Apparatus

4.1 Hot-plate.

4.2 Electric muffle furnace, with thermostat.

4.3 Crucibles for ashing: platinum or platinum and gold alloy (10% Pt, 90% Au), rectangular (60X40X25mm) or circular (diameter: 60 to 75mm, height: 20 to 25mm).

5. Procedure

The sample was ashed using the method for the determination of ash. The ash was transferred into a 250 to 400 ml beaker using 75 ml 3N hydrochloric acid (3.1) and evaporated to dryness. Heating was continued for at least one hour to dehydrate any silica which may be present and after cooling, 75 ml 3N Hydrochloric acid (3.1) was added and boiled gently for 15 minutes. The warm solution was filtered through an ash-free filter paper and the residue was washed with warm water until the filtrate was no longer acid. The filter was dried containing the residue and ash in a tared crucible (4.3) at a temperature of not less than 550°C and not more than 700°C, cooled in a desiccator and weighed.
6. Expression of the Results

The weight of the residue was calculated and the result was expressed as a percentage of the sample.

Fibre

1. Scope and Field of Application

This method is for the determination of the fibre content of feeding stuffs.

2. Principle

The sample is defatted and treated successively with boiling solutions of sulphuric acid and sodium hydroxide of specified concentrations. The residues is separated by filtration, washed, dried, weighed and ashed. The loss of weight resulting from ashing corresponds to the fibre present in the test sample.

3. Reagents

3.1 Sulphuric acid solution, 0.255N.
3.2 Sodium hydroxide solution 0.313N; the solution must be free or nearly free from sodium carbobate.
3.3 Anti-foam agent (e.g. silicone)
3.4 Ethanol, 95% (V/V)
3.5 Diethyl ether
3.6 Light petroleum, boiling range - 40-60°C
3.7 Hydrochloric acid: dilute 10 ml of hydrochloric acid (d=1.18g/ml) with water to 1000ml.

4 Apparatus

4.1 Conical flask, 1000ml.
4.2 Buchner flask
4.3 Bucher funnel
4.4 Platinum or silica crucibles
4.5 Electric muffle furnace.
4.6 Extraction apparatus for removal of fatty material
5: Procedure

Material was weighed to the nearest 0.0001 g. Between 2.7 and 3.0 g of the prepared sample was transferred to the extraction apparatus (4.6) and extract with light petroleum (3.6). Alternatively, extract with light petroleum by stirring, settling are at ordinary temperature and brought to boiling point, the first 30 to 40 ml being used to disperse the sample, heated to boiling point within 1 minute. An appropriate amount of anti foam agent (3.3) was added when it was necessary. Boiling was continued gently for exactly 30 minutes maintaining a constant volume and rotating the flask every few minutes in order to mix the contents and remove particles from the sides.

Meantime Buchner funnel was fitted with a suitable filter paper of such quality that it does not release any paper fibre during washings (it is convenient to use two filter papers, thereby facilitating the transfer of the insoluble matter at a later stage). Boiling water was poured into the funnel and was allowed to remain until the funnel was hot and then drained by applying suction.

At the end of the 30 minutes boiling period, the acid mixture was allowed to stand for 1 minute and then poured immediately into a shallow layer of hot water under gentle suction in the prepared funnel. The suction was adjusted so that the filtration of the bulk of the 200 ml is completed within 10 minutes. (The determination was repeated if time is exceeded).

The insoluble matter was washed with boiling water until the washings were free from acid, then washed back into the original flask by means of a wash bottle containing 200 ml 0.313N sodium hydroxide solution (3.2) measured at ordinary temperature and brought to boiling point. It was boiled for 30 minutes with the same precautions as those used in the earlier boiling and treatment and was allowed to stand for 1 minute and then filtered immediately through a suitable filter paper. The whole of the insoluble material was transferred to the filter paper by means of boiling water, it was first washed with boiling water then with dilute hydrochloric acid (3.7) and finally with boiling water until free from acid. Then it was washed twice with ethanol (3.4) and three times with diethyl ether (3.5). The insoluble matter was transferred to a dried, weighed, ashless filter paper and dried at 100°C to a constant weight and allowed to cool in a desiccator and weighed. The paper and insoluble matter was transferred to a crucible (4.4) previously ignited and content heated at a dull red heat to a constant weight and allowed to cool in dessicator and weighed.
6. Expression of the Results

The fibre content, as a percentage of the sample, is given by the formula:

\[ \frac{d - (p + a)}{W} \times 100 \]

where:

- \( d \) = weight of the paper + insoluble matter after drying (g)
- \( p \) = weight of the paper (g)
- \( a \) = weight of the ash (g) and
- \( W \) = weight of the sample (g)

3.9. Study of Post-harvest physiological (nutritional) changes in above ground biomass of *Cenchrus ciliaris.*

Nowadays, great emphasis is being laid on the evolvement of high-yielding nutritive and short-duration varieties of fodder crops. With the availability of high fodder yielding varieties of season bound and perennial fodder crops, there is a glut of fodder during the peak periods of growth and scarcity during other periods. **The best way to regulate the supply of palatable and nutritive fodder during the lean periods of October and November and May to July is to store the surplus fodder.**

A similar situation is also experienced in the case of grassland species which essentially comprise the monsoon grasses. These grasses give abundant fodder during the monsoon, but in the post-monsoon period and summer the forage production is almost negligible owing to their dormancy with the advent of winter and acute moisture stress. Thus it is essential that surplus fodder should be stored during the period of excess growth.

Above ground material was taken after harvesting and divided into two parts. Both the material were kept in the polythene bags and labeled. Both were kept at different temperatures. One was kept at room temperature at 20±2°C and another was kept in the refrigerator at 5±2°C. After two years of the storage of this material nutritional analysis was done. Estimations of crude protein, nitrogen, phosphorus and potassium was done. Statistical analysis of all data was done to evaluate significance of the treatments given.